EFFECT OF BIFENTHRIN ON MEMORY PROCESSES, MOVEMENT ACTIVITY, AND COORDINATION IN MICE EXPOSED TO TRANSIENT CEREBRAL OLIGAEMIA

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Abstract

The purpose of the present study was to examine whether the effects of exposure to 0.1LD₅₀ of bifenthrin on memory processes, movement activity, and coordination could be exacerbated by transient reduction of cerebral oxygen supply. The transient occlusion of both common carotid arteries (BCCA) in adult mice was performed under anaesthesia. Intraperitoneal LD₅₀ for bifenthrin was estimated to be 16.1mg/kg b.w. The memory retention was evaluated in a step-through passive avoidance task (PA), working spatial memory in a Y-maze, movement coordination on a rota-rod, and movement activity in an automated device. Long-term memory impairment caused by bifenthrin was exacerbated by BCCA. Movement co-ordination was significantly altered in animals treated with the compound. Movement activity was slightly decreased in animals after BCCA and pesticide injection. These results indicate that cerebral oligaemic hypoxia potentiates long-term memory impairing effect of bifenthrin.

Key words: mice, bifenthrin, memory processes, movement activity, transient cerebral oligaemia.

Pyrethroids are synthetic insecticides derived structurally from the natural pyrethrins: pyrethrin, cinerin, and jasmolin (16). The synthetic pyrethroids retain the potent and rapid insecticidal activity and low mammalian toxicity of natural pyrethrins but are more photostable. Therefore, they have found a widespread use in agricultural and public health applications. The low toxicity of pyrethrins and pyrethroids to mammals results from the fact that these substances are readily cleaved at the central ester bond to relatively non-toxic metabolites (3). The relative resistance of mammalians to these compounds is attributed to their higher body temperature than that of insects and low sensitivity of sodium channels, which are the target sites for pyrethroids (17).

Bifenthrin is a cyclopropanecarboxylate ester of alcohol. It has strong insecticidal properties due to the ability of altering functioning of insect nerves by modifying the kinetics of voltage-sensitive sodium channels (16). It was found to alter gait and other motor functions in rodents (16). Bifenthrin is one of the most commonly used pesticides. According to investigations of German researchers who examined human urine samples for metabolites of pyrethroids, the general population is universally exposed to these compounds (15). This means that elderly people experiencing transient ischaemic brain attacks (TIAs) are also exposed to the compound.

A transient occlusion of both common carotid arteries (BCCA) is an animal model reflecting the most typical features of TIAs (8). BCCA reduces blood flow in the brain but does not result in necrotic cell damage in its most vulnerable structures (13). However, it was found that BCCA causes hippocampal cholinergic dysfunction (7), increases GABA, dopamine, aspartame, glutamate, and hydroxyl radical levels in the brain (8). The procedure of BCCA was shown to impair memory processes (7).

In this paper we focused on bifenthrin and its neurotoxicity in mice. As cerebral oligaemic hypoxia is known to potentiate memory impairment induced by variety of chemicals (6, 9, 10), an interesting question arises: if it is able to produce any effects on memory and movement processes together with a pyrethroid pesticide, bifenthrin. Therefore we performed a set of neurobehavioral tests in order to denote any changes in memory acquisition, retention, spontaneous movement activity, and coordination following the intraperitoneal administration of a single dose bifenthrin and BCCA.

Material and Methods

Non-gravid female Albino-Swiss mice weighing 18-24 g, approximately 6 weeks of age, purchased from a licensed dealer (T. Górzkowski, Warsaw, Poland) were used in the study. All the animals were given a 7-d acclimation period and were
maintained on a 12L:12D photoperiod (0600:1800). Feed and tap water were provided ad libitum. The temperature was maintained at 21±2°C. All the experimental procedures were approved by the Local Ethics Committee for Animal Experiments.

There were four groups of ten animals each: I - BCCA-operated injected with 0.1 LD₅₀ bifenthrin i. p., II - BCCA-operated injected with biddistilled water i. p., III - sham-operated (with their carotids separated, but not clamped) injected with 0.1 LD₅₀ of bifenthrin i. p., and IV - sham-operated injected with biddistilled water i. p.

The surgical procedure of bilateral clamping of the BCCA was performed under ketamine (100 mg/kg b.w.) + xylazine (20 mg/kg b.w.) intraperitoneal anaesthesia. Ketamine (Ketanest, 50 mg/mL in 10 mL vials) was purchased from Parke-Davis, Germany. Xylazine (Rometar, 20 mg/mL in 50 mL vials) was purchased from Spofa, Czech Republic. The two anaesthetics were used in a combination as this mixture was successively used and evaluated before in horses, dogs, and cats (1, 12, 18). Xylazine is recommended as a sedative and anaesthetic to be administered systemically in mammals (14). Ketamine itself is known to produce sedative and anaesthetic to be administered systemically in mammals (14). Ketamine itself is known to produce decreased PaO₂, increased PaCO₂, blood pH decrease, cardiovascular system with disturbed ventilation, increased muscle tone, trembling, inhibition of the respiratory system, and body temperature decrease (2).

The mice were subjected to bilateral clamping of the BCCA by wrapping threads around the arteries to occlude blood flow for 30 min. The cessation of carotid blood flow was controlled visually. After 30 min the threads were removed, arteries were inspected for blood re-flow, and the surrounding skin was sutured. Sham-operated mice had their carotids exposed, but not clamped. During the procedure, the mice were breathing spontaneously and were kept at a constant temperature of 37°C by a heating pad and a lamp. Two to three hours after the procedure, they regained normal activity, ate and drank. Twenty-four hours after the surgery, groups I and III were injected with 0.1 LD₅₀ of bifenthrin i. p.

Bifenthrin (technical grade 99%) as pulvis in 0.25 g vials was purchased from the Institute of Industrial Organic Chemistry, Warsaw, Poland. Intraperitoneal LD₅₀ of bifenthrin was estimated with use of a computer programme based on Lichtfield and Wilcoxon's method (11). The estimated dose was 16.1 mg/kg b.w. (13.1-19.7). 0.1 LD₅₀ of bifenthrin was suspended in 10 mL of biddistilled water with two drops of Tween 20 (Laboratoriums Reagenzien, Germany).

Memory retention was examined in step-through passive avoidance task (PA). The task relies on the innate preference of rodents for dark, enclosed spaces and it is regarded as a measure of long-term memory retention. Thirty minutes after the bifenthrin or biddistilled water injection, each animal was placed in an illuminated box (15 x 12 x 15 cm) adjacent to a darkened one (the same size) with an electric grid floor. Thirty second after placing the animal in the centre of the illuminated box, a passage joining the two boxes was opened. After entering the dark box, the animal was affected with an electric foot shock (2 mA for 2s). Twenty-four hours after the training trial, memory retention test was conducted in which the same animals were placed in the illuminated box and the latency to enter the darkened box was recorded. The test ended when the mouse entered the darkened box or when 180 s elapsed.

Working spatial memory was assessed by recording spontaneous movement alternation in the Y-maze. The maze consists of three 10 x 10 x 10 cm boxes joined together with 4-cm long corridors at 120° in such a way that each corridor opens to one box only. The maze has no floor. It is placed on a clean sheet of paper on a tabletop. Mice tend to explore the maze by systematically entering each arm. The ability to alternate requires that the animals knew which arm they had already visited. In the task, each mouse was placed at the end of one arm and was allowed to move through the maze for 8 min percentage of alternation, defined as consecutive entries into all three arms without repetitions in overlapping triplet sets, to possible alternations x 100% was counted.

Movement coordination was examined next on a rotating rod (10 rotations/min). The animals were placed on the rotating rod (1 cm diameter) 50 cm above the ground for 120 s.

Spontaneous movement activity was measured then within 30 min in automated devices (boxes of 30 cm diameter), each equipped with two photocells and a counter.

A Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test was used to analyse the data from PA test. PA results were expressed as median values (with 25th and 75th percentiles). The results from the Y-maze, movement coordination test, and spontaneous motor activity test were shown as means ± SEM and evaluated by one-way ANOVA followed by Duncan's post hoc test for multiple comparisons. P<0.05 was considered statistically significant.

Results

The results obtained in the step-through passive avoidance task are shown in Fig. 1.

There were no statistically significant differences among groups I-IV in working spatial memory task in the Y-maze. The obtained results were as follows: group I - 55.5±2.945, group II - 56.74%±2.945, group III - 52.62%±2.945, and group IV - 62.4±1.6. Movement coordination was significantly impaired in groups I and II (treated with bifenthrin) vs group IV (control; sham-operated). BCCA was shown to exacerbate the effect (Fig. 2).

Spontaneous movement activity was slightly decreased in groups I and II (after BCCA) without statistically significant differences vs control. The results obtained in spontaneous motor activity were as follows: group I - 275.7±31.896, group II - 279.7±29.912, group III - 342.9±39.67, and group IV - 346.6±33.532.
Fig. 1. Results obtained in the step-through passive avoidance task. There were 10 animals in each group.

Fig. 2. Results obtained in movement coordination test on a rotating rod. There were 10 animals in each group.
Discussion

In the case of bifenthrin, the intraperitoneal route of administration is not directly relevant to human exposure but it was chosen in this study because of precise dosing possibilities. There are differences described between different studies on bifenthrin toxicity depending on routes of the administration of the chemical. In vast majority of publications, the pyrethroid was administered orally or by gavage rather than intravenously, rarely intraperitoneally (16).

Oral LD₅₀ of bifenthrin/kg b.w. for rats, estimated by Freeman (4), was 70.1 and 53.8 for males and females, respectively. However, intraperitoneal LD₅₀ of the toxins is known to be lower than the oral one.

There are available results of detailed studies on neurotoxicity of bifenthrin performed in accordance with the regulatory guidelines provided by the U.S. Environmental Protection Agency (EPA) (19). After administration of 75 mg/kg b.w. of bifenthrin per os to Sprague-Dawley rats, Watt (20) observed whole-body tremors, twitching, staggered gait, uncoordinated movement/ataxia, splayed hindlimbs, abnormal posture, clonic convulsions, and abdominogenital staining. No effects were evident either at the dose of 35 or 10 mg/kg b.w. Freeman (5) studied subchronic toxicity of bifenthrin administered per os to Sprague-Dawley rats. At the level of 100 and 200 ppm, bifenthrin produced whole-body tremors, twitching, decreased grip strength, and increased landing foot splay. At 50 ppm, no effects were evident.

The results obtained in the present study seem to be congruent with the above data, as bifenthrin was shown to impair movement coordination. Little is known about its influence on memory processes, but the results observed in passive avoidance step-through test in this study indicate clearly that bifenthrin is able to impair long-term memory retention, especially in individuals subjected to transient oligemic brain hypoxia.

In conclusion, even though there is a wide range of bifenthrin use in agricultural, veterinary, medical, and household pest control, it should be used with precautions, especially by elderly people, as it can impair memory retention and movement coordination in individuals subjected to transient reduction of cerebral oxygen supply.

References